

## Research Article

# Nectar Meals of a Mosquito-Specialist Spider

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*Evarcha culicivora*, an East African jumping spider, is known for feeding indirectly on vertebrate blood by actively choosing blood-carrying mosquitoes as prey. Using cold-anthrone tests to detect fructose, we demonstrate that *E. culicivora* also feeds on nectar. Field-collected individuals, found on the plant *Lantana camara*, tested positive for plant sugar (fructose). In the laboratory, *E. culicivora* tested positive for fructose after being kept with *L. camara* or one of another ten plant species (*Aloe vera*, *Clerodendron magnifica*, *Hamelia patens*, *Lantana montevideo*, *Leonotis nepetaefolia*, *Parthenium hysterophorus*, *Ricinus communis*, *Senna didymobotrya*, *Striga asiatica*, and *Verbena trivernia*). Our findings demonstrate that *E. culicivora* acquires fructose from its natural diet and can ingest fructose directly from plant nectaries. However, experiments in the laboratory also show that *E. culicivora* can obtain fructose indirectly by feeding on prey that have fed on fructose, implying a need to consider this possibility when field-collected spiders test positive for fructose. In laboratory tests, 53.5% of 1,215 small juveniles, but only 3.4% of 622 adult *E. culicivora*, left with plants for 24 hours, were positive for fructose. These findings, along with the field data, suggest that fructose is especially important for early-instar juveniles of *E. culicivora*.

## 1. Introduction

Trophic switching and feeding at more than one trophic level, although often overlooked in the literature on spiders, are common themes in the evolution of arthropods [1, 2]. For example, many predatory heteropterans are known to feed facultatively on plant products [3, 4]. Spiders, however, are typically characterized as being obligate predators. The most striking known exception is *Bagheera kiplingi* [5], a Central American jumping spider (Salticidae), which is almost entirely herbivorous despite cohabiting with edible ant species (*Pseudomyrmex* spp.). *B. kiplingi* feeds primarily on the Beltian bodies (specialized leaf tips) of the ant-acacia (*Vachellia* spp.), which also dominate the ants' diet [6–8]. Although no other spiders are known to rely as heavily on herbivory as *B. kiplingi*, many spiders do supplement a predatory diet with nectar taken from the floral or extrafloral nectaries of plants (e.g., [9–12]).

Taylor and Pfannenstiel [13] and Chen et al. [14] provided evidence of fructose ingestion by one or more species

from each of 13 spider families (Agelenidae, Anyphaenidae, Araneidae, Clubionidae, Corinnidae, Lycosidae, Miturgidae, Nephiliidae, Oxyopidae, Pisauridae, Salticidae, Tetragnathidae, and Thomisidae). Presence of fructose was confirmed using cold-anthrone testing, a procedure developed by Van Handel [15, 16] for detecting the presence of fructose in mosquitoes. While field and laboratory observations suggest that nectarivory might be especially prevalent among jumping spiders (Figure 1) [12, 17], only one species (*Plexippus selipes*) has been shown to be fructose positive by cold-anthrone testing [14].

Salticids have intricate vision-guided predatory strategies supported by their complex eyes [18–20], and the predatory strategy of *Evarcha culicivora* is unusually intricate even by salticid standards [21]. This species feeds indirectly on vertebrate blood by actively choosing blood-carrying female mosquitoes as preferred prey [22], a choice it can make even when restricted to using chemoreception alone. Olfactometer experiments [22, 23] have also shown that *E.*



FIGURE 1: *Evarcha culicivora* juvenile approaching nectar on an extrafloral nectary of *Ricinus communis*.

*culicivora* is attracted to the odour of two plant species, *Lantana camara* and *Ricinus communis*, but the role of these plants in *E. culicivora*'s biology remains largely unknown.

Here we investigate whether *E. culicivora*'s attraction to *L. camara* and *R. communis* can be explained, at least in part, by the spider acquiring nectar meals from these plants. Using cold-anthrone testing, we confirm that some of the *E. culicivora* individuals collected from *L. camara* in the field have ingested fructose. We then repeat cold-anthrone testing under laboratory conditions to minimize the possibility of the spiders acquiring fructose by any means other than feeding directly on the plant's nectaries, such as feeding on other parts of the plant or on fructose-carrying prey (see: [14, 24]). Finally, we determine the specificity of *E. culicivora*'s interest in particular plants by testing for the presence of fructose in individuals that had been housed with one of ten other plant species.

## 2. Materials and Methods

**2.1. General.** Our field site was the Thomas Odhiambo Campus (Mbita Point) of the International Centre of Insect Physiology and Ecology (ICIPE) in Western Kenya (elevation 1200 m above sea level; latitude 0°25'S–0°30'S; longitude 34°10'E). For the rearing and maintenance of spiders in the laboratory, we followed procedures that are standard for our salticid research (see: [25]) and summarize only essential details here.

The laboratory photoperiod was 12L:12D, with lights coming on at 07:00 am. Except for recently hatched juveniles (see below), each individual spider was maintained in a standard cylindrical cage (diameter 45 mm, height 55 mm) made of transparent plastic with two holes in the top (a screen-covered hole for ventilation and another hole used for introducing prey). Each spider had continuous access to water in its cage via a cotton roll that protruded through a hole in the bottom of the cage into a water-filled pot below. All holes were 10 mm in diameter. The spiders were maintained on a mixed diet of non-biting

midges (Chironimidae) collected as needed from the field and blood-fed female mosquitoes (*Anopheles gambiae* s.s.) from cultures (see: [26]). The spiders were provided with these prey three days per week (Monday, Wednesday, and Friday).

**2.2. Cold-Anthrone Testing.** No later than 4 hours before use (see: [27]), a fresh batch of anthrone reagent was prepared by mixing 150 mL of distilled water with 380 mL of concentrated sulphuric acid, after which 150 mg of anthrone powder was mixed with 100 mL of the diluted sulphuric acid.

Each spider from the field or from an experimental trial in the laboratory (see below) was placed in a vial and stored at  $-80^{\circ}\text{C}$  to arrest enzymatic activity. After 4 hours, the frozen spider was removed and transferred to a 5 mL test tube. Moisture was evaporated off the spider by holding the test tube in a hot water bath ( $80\text{--}90^{\circ}\text{C}$ ) for 15 minutes (see [15]). The next step in preparing the spider for cold-anthrone testing was to remove cuticular wax and expose the spider's digestive tract. This was achieved by using a solution of chloroform and methanol (ratio of 1 : 1), which had been prepared ahead of time and stored at  $-25^{\circ}\text{C}$ . Two drops of this solution were added to the test tube with the spider. 20 minutes later, the spider was gently crushed using a glass stirring rod.

Next, 0.5 mL of the anthrone reagent was added to the test tube, which was then agitated for 60 minutes on a vortex mixer held at  $26^{\circ}\text{C}$  in a water bath. We followed established procedures for preparing colorimetric standards corresponding to different fructose concentrations [28]. These standards were made by pipetting  $1\ \mu\text{L}$  of each of nine standard sucrose solutions (see below) into test tubes (one test tube per standard) and adding two drops of the chloroform-methanol solution and 0.5 mL of anthrone reagent. The initial sucrose solution was made by dissolving 25.6 g of reagent grade sucrose in 50 mL of distilled water and adding enough water to make 100 mL of solution. Next, we made eight two-fold serial dilutions ("standards"), as explained by Taylor and Pfannenstiel [13], each standard corresponding to a specified concentration of fructose. Standards were stored at  $-45^{\circ}\text{C}$ .

Samples from cold-anthrone testing of spiders were evaluated by visual inspection for colour change. When fructose was present, samples turned green or blue green, but samples lacking fructose remained clear yellow. We adopted matches to the standards at above  $2\ \mu\text{g}$  as our criterion for recording a sample as being positive for fructose. This criterion was derived from "sponge tests" (see below) designed to determine how effective our cold-anthrone methods were at detecting fructose specifically in spiders (i.e., we determined the threshold match to sample above which glucose would not give a false positive for fructose). Accordingly, estimates for how many spiders ingested fructose should be envisaged as conservative. Considerable digestion of fructose might have occurred during the interval between the spider ingesting nectar and the spider being transferred to a freezer ( $-80^{\circ}\text{C}$ ), and this is another factor suggesting that our estimates of numbers of spiders that ingested fructose are conservative.

**2.3. Sponge Testing.** Earlier research [29] has shown that sponge discs soaked in honey solutions can be used for supplementing the diet of spiders. Here we used sponge discs to provide *E. culicivora* juveniles with opportunity to feed on nectar in the absence of plants. To initiate a sponge test, a clean disc (diameter 5 mm, thickness 2 mm) cut from a rubber sponge was dipped in a vial containing nectar or a sugar solution (30% fructose or 30% glucose) for 10 seconds, then transferred to a clean rearing cage. There was a cork, rather than a cotton roll, in the hole in the bottom of the cage and the disc was pinned to the inside end of this cork. A spider was put into the cage at 08:00 am and a 1-hour or a 24-hour individual test (see above) was carried out. There were no plant cuttings in the cage.

The nectar came from *Leonotis nepetaefolia* grown in a field plot. We used this plant species because its flowers produce copious volumes of nectar. Nectar was squeezed by hand into plastic vials (diameter 10 mm; height 48 mm), after which the vials were stored in a freezer at  $-25^{\circ}\text{C}$ . We discovered that nectar volume was usually low in the afternoon, probably due to depletion by nectarivorous birds and insects. We avoided this problem by collecting early in the morning (06:00–07:00 am).

#### 2.4. Testing Spiders for Fructose after Being Housed with Plants.

In the field, we collected individuals of *E. culicivora* that we found on the flowers of a particular plant species, *Lantana camara*, and, within 60 minutes, transferred each collected spider to a freezer ( $-80^{\circ}\text{C}$ ) in preparation for cold-anthrone testing. The rationale for the focus on *L. camara* was partly that it is one of the two plants known to attract *E. culicivora* [23] and partly that it is one of the most common plant species in our field site.

For laboratory testing, we used *L. camara* and *Ricinus communis*, the two plant species known to attract *E. culicivora* [23], as well as another nine species chosen as an arbitrary sample of the numerous plants present in the study site (see [30]). Plant cuttings collected from the field were held in a closed plastic box under 100% carbon dioxide for 10 minutes and then examined carefully with a microscope for any arthropods (e.g., plant-eating insects) that might have remained on the plant. None were found. Next, the plant cutting was put into a cage (the size of the cutting was sufficient to almost fill the cage). The cut stem at the bottom of the cage was wedged next to the cotton roll and extended into the water in the pot below the cage, while the rest of the cutting (flowers, stems, and leaves) was within the cage. Testing began at 08:00 am, when spiders were introduced into cages. We decided not to consider differences in how the plants responded to the treatment (e.g., drying out with exposure to  $\text{CO}_2$ ) because we were primarily interested in determining qualitatively whether the spiders ingest any nectar at all from the various plants.

In the laboratory, *E. culicivora* females put their eggs in silk egg sacs situated inside cocoon-like silk nests. To acquire the juvenile spiders used for testing in the laboratory, females were removed from their cages on the day eggs were laid. After the eggs hatched and the juveniles emerged

from the nest, we waited 3 days before using these juveniles in experiments. The juveniles we used had not yet fed before testing. By using recently emerged unfed juveniles, we eliminated the possibility of these spiders having acquired fructose indirectly by feeding on insects that had been feeding on plants. A 3-day waiting period was adopted because after longer fasting periods juveniles often appeared weak and, after more than 3 days, many of these spiders died. For laboratory testing, we also used adult spiders that had matured 3–4 weeks before use. Adult spiders had not mated and were fasted for 7 days before testing.

For testing spiders with plants, three protocols were adopted: 24-hour communal testing (juveniles only, all plant species), 24-hour individual testing (adults only, all plant species), and 1-hour individual testing (juveniles only, *L. camara*, *R. communis*, and *L. nepetaefolia* only). All testing began at 08:00 am. For 24-hour testing (communal and individual), spiders were left in cages with plants until 08:00 am on the following day. Communal testing included a group of about 20 spiders per cage and individual testing included only one spider per cage.

Directly observing the behaviour by which spiders acquired fructose was not part of the protocol for field-collected spiders or during 24-hour testing in the laboratory. However, we defined “feeding on nectar” as instances of the spider having its mouth-parts pressed against floral or extra-floral nectar and, by this definition, we saw spiders feeding on nectar during casual observations. We saw no instances of the spider having its fangs extended or making back and forth movement of chelicerae (i.e., no biting was seen).

The procedure adopted for 1-hour individual testing was to place one spider directly on the plant and then observe it continuously. Testing ended when the spider stopped feeding (i.e., when it moved its mouthparts away from the nectar for 60 seconds). We aborted the test whenever an individual had not initiated feeding after 60 minutes had elapsed. This procedure meant that, in 1-hour individual testing, we were certain the spiders we assayed using the cold-anthrone method had, according to our definition, fed on nectar and that there was no alternative means by which these spiders might have acquired fructose (i.e., none were seen with fangs extended or chelicerae making biting movements, and none were seen feeding on prey).

#### 2.5. Mosquitoes as an Indirect Source of Fructose for Spiders.

For normal rearing, mosquitoes were given access to a 6% glucose solution soaked into cotton wool (see: [26]). For our experiments, instead of the normal 6% glucose solution, we used female mosquitoes that had been given access to a 6% fructose solution (via a sponge disc that had been soaked in the fructose solution). None of these mosquitoes had been fed blood. We kept each mosquito in a separate cage with a sponge disc. This was preferable to trying to feed fructose to mosquitoes in a group, as competition for access to food would have made it difficult to ensure that most mosquitoes would receive a fructose meal during the feeding period. At 08:00 am on the following day, these fructose-fed mosquitoes were put with the spiders (each juvenile spider in a separate

cage). 24 hours later, the spider was transferred to a freezer ( $-80^{\circ}\text{C}$ ) in preparation for cold-anthrone testing.

## 2.6. Statistical Methods

**2.6.1. Field-Collected Spiders.** We measured the body size (accurate to the nearest mm) of 95 field collected individuals before testing them for fructose. We then conducted a logistic regression analysis [31] and compared the resulting model to a constant only model to determine whether body size was an accurate predictor of fructose presence. We calculated Nagelkerke's  $R^2$  [31] to assess the strength of this association and the Wald criterion [31] to determine the degree to which the predictor contributed to the strength of the model. Finally the odds ratio [31] was calculated to show the magnitude of change across the regression.

**2.6.2. Spiders Housed with Plants or Mosquitoes.** When one or more adults tested positive for fructose we conducted a  $\chi^2$  test of independence [31] to compare results of the fructose tests between males and females. We conducted a further series of  $\chi^2$  tests to compare the results of the fructose tests between adult and juvenile spiders.

All statistical tests were run using PASW Statistics software [32].

## 3. Results

**3.1. Presence of Fructose in Field-Collected Spiders.** As body size of the spiders sampled from the field increased, fewer individuals tested positive for fructose (Table 1). A test of the full model from the logistic regression against a constant only model was statistically significant, indicating that the predictor reliably distinguished between individuals that had consumed fructose and those that had not ( $\chi^2 = 10.455$ ,  $P < 0.001$ ,  $df = 2$ ). Nagelkerke's  $R^2$  was 0.168, indicating a weak relationship between prediction and grouping. Prediction success overall was 81.1%. The Wald criterion demonstrated that body size made a significant contribution to prediction ( $\chi^2 = 7.876$ ,  $P = 0.005$ ). The EXP(B) value indicated that when body size is raised by one unit (1 mm) the odds ratio becomes 0.461 times as large.

**3.2. Sponge Testing.** 28 out of 35 spiders were positive for fructose after being left for 24 hours with the sponge pieces that had been soaked in a fructose solution. Three of 35 spiders left with sponge pieces that had been soaked in a glucose solution were positive after cold-anthrone testing. These samples matched the  $2\ \mu\text{g}$  standard. Based on these findings, we required a match to standard above  $2\ \mu\text{g}$  as our criterion for recording that a spider was positive for fructose (i.e., our data from sponge testing suggest that match to a sample of  $2\ \mu\text{g}$  cannot be distinguished from a false positive).

Although continual observation was not part of the 24-hour testing protocol, we frequently saw spiders with their mouthparts pressed against the damp pieces of sponge during casual observation. 40 out of 102 spiders were observed feeding during 1-hour continual observation trials. 37 of

TABLE 1: Cold-anthrone results from testing field-collected *Evarcha culicivora* individuals of different sizes. All spiders collected from the plant *Lantana camara*.

Spider body length (mm)	Number positive for fructose (% positive for fructose)
2 mm	10 of 29 (34.5%)
3 mm	5 of 22 (22.7%)
4 mm	2 of 18 (11.1%)
5 mm	1 of 19 (5.3%)
6 mm	0 of 7

those 40 spiders subsequently tested positive for fructose. All spiders that were not seen feeding tested negative for fructose.

**3.3. Presence of Fructose in Spiders Housed with Plants or Mosquitoes.** Only 21 out of 622 (3%) adult spiders tested negative for fructose after being housed with a plant cutting for 24 hours. The small number of spiders that tested positive had been housed with *Aloe vera*, *Leonotis nepetaefolia*, or *Ricinis communis*. A series of  $\chi^2$  tests comparing results between males and females for each of these groups showed no significant difference between adults of the two sexes (Table 2). Accordingly, data from adult males and females were pooled before being compared with data from juveniles. For each plant species used, juveniles tested positive for fructose significantly more often than adults (Table 3) after being housed with a plant cutting for 24 hours.

When housed with a nectar source and observed continually for 1 hour, those individuals that were seen with their mouthparts on the plant nectaries almost always tested positive for fructose (Table 4). Spiders were never observed feeding from parts of the plant other than the nectaries.

In the absence of plants or sugar on sponge pieces, 19 of 57 (33%) spiders tested positive for fructose after feeding on fructose-carrying mosquitoes.

## 4. Discussion

Findings from cold-anthrone testing of field-collected *E. culicivora* suggest that ingesting fructose is characteristic of this spider species. As in other studies in which spiders from the field have been sampled for fructose [13, 14], we could not rule out the possibility that our spiders from the field fed from some part of the plant other than the nectaries or that they acquired fructose indirectly by feeding on fructose-carrying prey. However, our laboratory data support our hypothesis that spiders in the field acquire fructose primarily by taking nectar directly from the plants' nectaries.

Owing to pretesting procedures, which should have removed most potential prey from the experimental plants, it is unlikely that instances of spiders being positive for fructose after 24-hour tests in the laboratory were the result of indirect acquisition of fructose from prey. Moreover, we can be especially confident that fructose was not acquired by means other than feeding directly from nectaries during the

TABLE 2: Intersexual comparisons of the numbers of *Evarcha culicivora* adults positive for fructose (cold-anthrone testing) after having been left with plants for 24 hours. There were no positive results for 8 of the 11 tested plant species, so these results are omitted.

Plant species	Females positive for fructose (% positive for fructose)	Males positive for fructose (% positive for fructose)	$\chi^2$ Test for independence, $\alpha = 0.05$
<i>Aloe vera</i>	1 of 40 (2.5%)	0 of 37	$\chi^2 = 0.937, P = 0.333$ ns
<i>Leonotis nepetaefolia</i>	5 of 35 (14.3%)	1 of 33 (3.0%)	$\chi^2 = 2.675, P = 0.102$ ns
<i>Ricinus communis</i>	6 of 35 (17.1%)	10 of 48 (20.8%)	$\chi^2 = 0.177, P = 0.674$ ns

TABLE 3: Number of *Evarcha culicivora* (juveniles and pooled data for adult females and males) that were positive for fructose (cold-anthrone testing) after being left with plants for 24 hours. Ranked from highest to lowest percentage positive for juveniles.

Plant species	Juveniles positive for fructose (% positive for fructose)	Adults positive for fructose (% positive for fructose)	$\chi^2$ Test for independence, $\alpha = 0.05$
<i>Lantana montevideo</i>	39 of 45 (86.7%)	0 of 29	$\chi^2 = 53.139, P < 0.001$
<i>Lantana camara</i>	155 of 195 (79.5%)	0 of 109	$\chi^2 = 176.771, P < 0.001$
<i>Clerodendron magnifica</i>	43 of 62 (69.3%)	0 of 31	$\chi^2 = 39.990, P < 0.001$
<i>Striga asiatica</i>	26 of 43 (60.5%)	0 of 25	$\chi^2 = 24.474, P < 0.001$
<i>Ricinus communis</i>	85 of 140 (60.7%)	16 of 83 (19.3%)	$\chi^2 = 36.106, P < 0.001$
<i>Leonotis nepetaefolia</i>	44 of 81 (54.3%)	4 of 68 (5.9%)	$\chi^2 = 39.719, P < 0.001$
<i>Verbena trivernia</i>	75 of 149 (50.3%)	0 of 24	$\chi^2 = 21.326, P < 0.001$
<i>Senna didymobotrya</i>	38 of 77 (49.3%)	0 of 61	$\chi^2 = 41.543, P < 0.001$
<i>Aloe vera</i>	68 of 184 (37.0%)	1 of 77 (1.3%)	$\chi^2 = 35.490, P < 0.001$
<i>Parthenium hysterophorus</i>	51 of 154 (33.6%)	0 of 89	$\chi^2 = 37.303, P < 0.001$
<i>Hamelia patens</i>	26 of 85 (30.6%)	0 of 26	$\chi^2 = 10.386, P = 0.001$

TABLE 4: Number of *Evarcha culicivora* juveniles that were observed feeding and number that were positive for fructose (cold-anthrone testing) after being left with plants for 1 hour. Spiders not seen feeding were never positive for fructose.

Plant species	Number seen feeding (% seen feeding)	Positive for fructose (% positive for fructose)
<i>Lantana camara</i>	12 of 25 (48.0%)	10 of 12 (83.3%)
<i>Ricinus communis</i>	18 of 32 (56.3%)	17 of 18 (94.4%)
<i>Leonotis nepetaefolia</i>	10 of 45 (22.2%)	10 of 10 (100.0%)

1-hour tests, as there was continuous observation. None of these spiders were ever seen feeding on prey or feeding on any part of a plant other than the nectaries and almost every spider that was observed feeding on nectaries subsequently tested positive for fructose.

From these data, we can confidently conclude that *E. culicivora* has the capacity to ingest nectar directly from nectaries. However, after having access to mosquitoes that had been feeding on a fructose solution, many *E. culicivora* juveniles tested positive for fructose and, in these tests, the mosquito was the only fructose source that could account for the findings. This result suggests that indirect fructose acquisition should be considered as a potential contributor to our fructose-positive results when field-collected spiders were sampled. Further research is needed to determine the relative importance of direct and indirect ingestion of plant-derived nutrients by *E. culicivora*.

Examining data from field-collected spiders, we found a negative relationship between the spider's size and whether it was positive for fructose. Fructose-positive results were also considerably more common for juveniles than for adults in the 24-hour laboratory tests. Although a number of factors, such as differential fructose metabolism and how the total amount of fructose ingested is related to the spider's body size, may also play a part in explaining these results, perhaps the most interesting hypothesis suggested is that nectar meals are especially important for the smaller juveniles. As we are currently investigating this hypothesis, here we will only mention some of the factors that might be particularly relevant.

Optimal foraging models often use energy intake as a proxy for the fitness benefits gained by feeding [33]. However, numerous examples [34], including some from studies on spiders [35, 36], show that nutrient regulation, not energy maximisation, may be the more important function of feeding. Perhaps nectar meals are more relevant to the optimal nutrient balance for small juveniles than for larger *E. culicivora* individuals. Furthermore, it may be that the volume of nectar readily acquirable from *L. camara* is large enough to be significant to small juvenile *E. culicivora*, but too small to be considered by larger individuals [37, 38].

The type of benefit gained by small juveniles from nectar may also be important. Although nectar does contain other nutrients, such as amino acids, its primary component is sugar [39, 40]. Our results may indicate that sugar meals are more important to small *E. culicivora* than they are to larger individuals. Early-instar spiders are more vulnerable

to starvation than their later-instar counterparts [41, 42], which may make easily acquired sugar meals more beneficial to small juveniles than they would be to larger juveniles or adults. A sugar meal may act to sustain a small juvenile long enough that it can succeed at capturing prey and thereby acquire a more nutrient-rich meal.

Earlier olfactometer experiments [23] showed that the odours of two plant species, *L. camara* and *R. communis*, attract *E. culicivora*. Nectar meals from these plants might be particularly important, but we have shown that *E. culicivora* can acquire nectar meals not only from these two plant species but also from each of the nine other plants used in our experiments. The full significance of *L. camara* and *R. communis* to *E. culicivora* may include more than just providing nectar meals. One of our goals in ongoing research is to fully investigate the role of particular plants in *E. culicivora*'s biology.

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